

Lymphocyte reactivity in patients with gonococcal urethritis

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SUMMARY Lymphocyte reactivity to virulent gonococcal antigen T₂ and the non-pathogenic *Neisseria pharyngis* (NPN) has been studied by using the ¹⁴C-thymidine uptake in cell cultures from 42 patients with gonococcal urethritis and from 18 controls. The DNA synthesis in cell cultures with T₂ antigen was higher in 21 female patients than in the 18 controls. No differences in DNA synthesis were observed in antigen-stimulated cell cultures from patients with single or multiple infections, from patients with urogenital complications, or from controls. Gonococcal antibodies in the serum were detected by the gonococcal complement-fixation test (GCFT). A study of the possible correlation between the outcome of the serological test and the cellular response to gonococcal antigen showed that ¹⁴C-thymidine uptake in lymphocyte cultures from male patients with negative GCFT, stimulated with T₂ antigen, was much lower than the thymidine uptake in stimulated cell cultures from all the other male and female patients ($P < 0.001$). The DNA synthesis was higher in cell cultures from seronegative women than from seronegative men ($P < 0.01$). A significant difference ($P < 0.01$) was also noted in the lymphocyte reactivity to gonococcal antigen between controls and all patients, except in those men who gave negative results to the serological tests. There were no differences between these two groups with respect to the thymidine uptake in NPN-stimulated cell cultures.

Introduction

Various immunological techniques to detect serum antibodies in gonococcal infection have been described. The gonococcal complement-fixation test (GCFT) is the most widely used test in the serodiagnosis of gonorrhoea (Sandström and Danielsson, 1975), and the only method used routinely. With this test sera from about 30% of patients with uncomplicated genital gonorrhoea and 50% of patients with salpingitis and genital gonococcal infections give positive results in a single serum specimen (Danielsson *et al.*, 1972). Non-specific reactions are found in about 5% of uninfected patients but are of little importance in patients with venereal diseases (Magnusson and Kjellander, 1965).

The cellular response to gonococcal antigen in patients with gonococcal urethritis has been studied *in vitro* using the lymphocyte transformation test

(Kraus *et al.*, 1970; Esquenazi and Streitfeld, 1973; Grimble and McIlmurray, 1973; Kearns *et al.*, 1973). This cellular response was not demonstrable in cells from controls but it was present in varying degrees in patients suffering from gonococcal urethritis. Antigen-induced lymphocyte transformation was only slightly greater in patients with a first infection than in controls, while the difference was often significant between patients with a history of at least two gonococcal infections and controls (Kearns *et al.*, 1973). In 1972 Rosenthal found no differences in lymphocyte reactivity to gonococcal antigen between patients with newly contracted gonorrhoea and controls. In 1975 it was demonstrated (Rosenthal, 1975) that lymphocyte stimulation to gonococcal antigen occurred in patients with joint complications after gonococcal infection, while no lymphocyte stimulation could be demonstrated in patients with gonococcal urethritis (either single or multiple infections) or in the controls.

The present investigation was undertaken to find out whether the discrepancy between our results

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and those of previously published studies may be due to the composition of the material or to the involvement of the urogenital tract in the form of prostatitis or salpingitis. The object was also to study the correlation between both the cellular and the humoral immune response.

Materials and methods

PATIENTS AND CONTROLS

Forty-two Caucasian patients were investigated; of these 27 were considered to have uncomplicated gonococcal urethritis and 15 had gonococcal urethritis with urogenital complications. Seventeen of the 27 patients (11 women and six men) had an infection for the first time which will here be referred to as single infection, and 10 had had multiple infections. Of the latter 10 patients, eight (two women and six men) had had only one previous episode of uncomplicated gonococcal infection. This episode had occurred between four months and seven years before examination. One man had had two previous gonococcal infections, and another had had several infections during a period of three and five years, respectively. The mean age of these 27 patients was 22.9 years; for the women it was 20.5 and for the men 25.3 years. The duration of symptoms was short (one and three days) in two of 13 female patients. Three women visited the hospital as contacts of patients with gonorrhoea. The other eight women had had a discharge for several months before the diagnosis was established.

Of 14 men, six with single gonococcal infection had had symptoms lasting a few days; one of these was symptom-free at the time of investigation. In the other eight men, who had had multiple gonococcal infections, the history of the current infection was also short, varying between one day and three weeks. Blood specimens from the patients with uncomplicated gonorrhoea were obtained at the time of diagnosis.

Of the above-mentioned 42 patients, 15 had local complications. Of these, eight were women with salpingitis (mean age 21.4), and seven were men with prostatitis (mean age 24.9). Seven of the women with salpingitis, who had cervical cultures positive for gonococci, had been referred from the gynaecological department with this diagnosis. Five of the eight women had salpingitis for the first time, and three had had previous episodes thought to be caused by gonococci. The interval between the diagnosis of infection and investigation was 33.9 ± 21.9 days. The duration of symptoms was short (less than one month) in six patients, whereas it was two months in the remaining patient.

Five of the seven male patients were diagnosed as having acute prostatitis because of localised swelling and tenderness of the prostate gland on rectal palpation. These changes disappeared after treatment. The duration of symptoms in all these cases was less than one month. Two men had had chronic prostatitis for the last two and three years.

The mean interval between the diagnosis of current infection and investigation was 26 days with a range of 0–90 days. Blood specimens from the patients with urogenital complications were obtained in the convalescent period of the disease (after antibiotic treatment) in 12 out of 15 patients. In all cases but one the diagnosis of gonococcal infection rested on the culture of oxidase-positive, Gram-negative diplococci fermenting glucose but not maltose or laevulose. The remaining patient, a man, was diagnosed by the finding of intracellular diplococci in a smear of urethral pus, stained with methylene blue.

Eighteen blood donors, 13 men and five women, served as controls. Data on age and race were available for 13 men and two women, with a mean age of 38.5 and 25.0 years, respectively. The controls were all Caucasian.

GNOCOCCAL COMPLEMENT-FIXATION TEST

Sera were collected at the same time as blood sampling for the lymphocyte transformation test and were stored frozen at -20°C until tested. Sera were thawed, inactivated at 56°C for 30 minutes, and analysed by the micromodification of the laboratory branch complement fixation test (US Public Health Service, 1965) as modified by Danielsson *et al.* (1972). Antigen was produced from T_2 colonies of five strains collected in 1973 from patients with uncomplicated gonococcal infection and grown on gonococcal special agar for 22 hours at 36°C in 5% CO_2 atmosphere (Sandström and Danielsson, 1977). Each of the strains was collected in saline, adjusted to a concentration of 25 mg/ml wet weight of bacterial cells in veronal buffer, heated to 60°C for 30 minutes, and tested in checkerboard titrations for anticomplementary activity and optimal dilution against a human serum with a known titre. The strains could be mixed in equal proportions to a final concentration of 1.0 mg/ml. The dilution of a serum specimen that gave an inhibition of haemolysis of 70% or more was regarded as the final titre. Such an inhibition at a serum dilution of 1:5 was regarded as a positive reaction.

LYMPHOCYTE TRANSFORMATION TEST

Lymphocyte separation

Lymphocytes were isolated from heparinised venous blood by flotation of blood on Ficoll-Isopaque (Lymphoprep, Nyco, Oslo, Norway), as described by Bøyum (1968).

The interface cell layer was removed and washed twice in phosphate-buffered saline (PBS). The cells were resuspended in Eagle's medium, in Earle's solution (Flow Laboratories, Irvine, Scotland) containing glutamine (2 mmol/l), 100 iu/ml penicillin, and 100 µg/ml streptomycin. To this medium was added 10 mmol/l N-2-hydroxy-ethylpiperazine-N'-2-ethane sulphonic acid (HEPES) buffer (Flow Laboratories). For induction of DNA synthesis the medium was supplemented with nonessential amino-acids and pyruvate as described by Mishell and Dutton (1967). The cells were cultured in this medium, with 10% heat-inactivated human AB serum added. Triplicate cell cultures were set up in plastic tissue culture tubes (no. 2058 Falcon Plastics) containing 0.5 ml lymphocyte suspension (3×10^5 cells/tube). To the cell cultures 0.05 ml of the following bacterial antigens and mitogen were admixed: *Neisseria gonorrhoeae* (strain 819) of colony morphology type T₂ and *Neisseria pharyngis* (strain 6131) at a concentration of approximately 2×10^6 organisms/tube, 0.05 ml of PPD tuberculin, 0.01 mg/ml and 0.05 ml of phytohaemagglutinin (PHA) 1:100. A control culture without antigen was made in each case. The culture tubes were incubated at 37°C in humidified 5% CO₂ in air atmosphere.

Measurements of DNA synthesis

A concentration of 0.1 µCi ¹⁴C-thymidine (specific radioactivity 25 mCi/mmol per litre, the Radiochemical Centre, Amersham) in 0.05 ml of PBS was added to the cultures with antigens and unstimulated cultures on day 5 and to cell cultures with PHA on day 2. The cultures were harvested 24 hours later using distilled water, and the cells were collected on Sartorius membrane filters. The filters were placed in scintillation plastic vials and left to dry at room temperature overnight. Five millilitres of toluene-based scintillation fluid were added to each vial, and the radioactivity was measured in a Intertechnique (Nanoteknik, Sweden) scintillation counter. The results were expressed as arithmetic means of counts/min of triplicate tubes \pm SE.

BACTERIAL ANTIGENS

The following bacterial strains were used: one strain of *N. gonorrhoeae* (laboratory designation 819) whole cells of colony morphology type T₂ (virulent) (Kellogg *et al.*, 1963) and one strain of *N. pharyngis* (designation 6131) whole cells. The

bacterial antigens were prepared by Dan Danielsson, Örebro, Sweden. The procedures used have been described in detail elsewhere (Rosenthal and Danielsson, 1978). PPD tuberculin was obtained from Statens Seruminstitut, Copenhagen, as a solution of 1 mg/ml in phosphate buffer, without addition of preservative.

Mitogen

PHA, obtained from Wellcome Foundation, was used in a final dilution of 1:100.

STATISTICS

The mean values are given in log transformed counts/min \pm SE. Student's *t* test was used to estimate statistical significance.

Results

The lymphocyte reactivity to the pathogenic (T₂) and non-pathogenic *Neisseria* (NPN) antigens was studied in 42 patients with uncomplicated and urogenitally complicated gonococcal urethritis and in 18 controls. The results are summarised in Table 1. The lymphocyte response showed no significant differences between patients and controls or between T₂-antigen stimulated and NPN-antigen stimulated cell cultures. The lymphocyte reactivity was therefore analysed in accordance with the following criteria: (a) number of gonococcal infections, (b) presence of urogenital complications, (c) serological response in the GCFT, and (d) sex distribution; this lymphocyte reactivity was compared with that in the controls. The patients with gonococcal urethritis were subdivided into three groups: single infection, multiple infection, and urogenital complications in the form of prostatitis and salpingitis (Table 2). The thymidine uptake in stimulated cell cultures was nearly the same in all the groups of patients. There was, however, a significant difference in the gonococcal-antigen-stimulated DNA synthesis between the 21 female

Table 1 T₂-induced and NPN-induced DNA syntheses in cells from 42 patients with gonococcal urethritis and from 18 blood donor controls

	10 log counts/min \pm SE (antilogs within parentheses)	
	T ₂	NPN
Patients	3.757 \pm 0.069 (5716)	3.885 \pm 0.065 (7673)
Controls	3.622 \pm 0.090 (4185)	3.933 \pm 0.084 (8570)
<i>t</i> test between patients and controls	<i>t</i> = 1.12 (<i>P</i> > 0.05)	<i>t</i> = 0.42 (<i>P</i> > 0.05)

Table 2 Induction of DNA synthesis by T_2 and NPN antigens in lymphocytes from 42 patients with gonococcal urethritis and from 18 blood donor controls

Diagnosis	No. of patients	10 log counts/min \pm SE (antilogs within parentheses)	
		T_2	NPN
Single infection	17	3.824 \pm 0.073 (6669)	3.949 \pm 0.092 (8892)
Multiple infections	10	3.579 \pm 0.164 (3793)	3.729 \pm 0.143 (5357)
Urogenital complications	15	3.800 \pm 0.135 (6309)	3.916 \pm 0.117 (8241)
Controls	18	3.622 \pm 0.090 (4185)	3.933 \pm 0.084 (8570)

patients and the 18 controls, t values being 2.40 $P < 0.05$ for log counts/min. No difference was observed between the 21 male patients and the controls (Table 3).

The GCFT was found to be positive in 12 of the 42 patients (Table 4). A study of the possible correlation between the outcome of the serological test and the results of the cellular reactivity to gonococcal antigen gave the following results (Figure). The ^{14}C -thymidine uptake in lymphocyte cultures from the 16 male patients with negative GCFT stimulated with T_2 antigen was much lower than the thymidine uptake in stimulated cell cultures from the other 26 male and female patients (t value = 4.01 $P < 0.01$). The difference was significant between the five seropositive and the 16 seronegative men (t value = 3.03 $P < 0.01$), but not between the seven seropositive and 14 seronegative women (t value = 0.20). The DNA synthesis

Table 4 Presence of complement-fixing antibodies in different patient groups

Diagnosis	Male patients		Female patients	
	Positive GCFT	Negative GCFT	Positive GCFT	Negative GCFT
Single infection	2	4	6	5
Multiple infections	1	7	0	2
Urogenital complications	2	5	1	7
Total	5	16	7	14

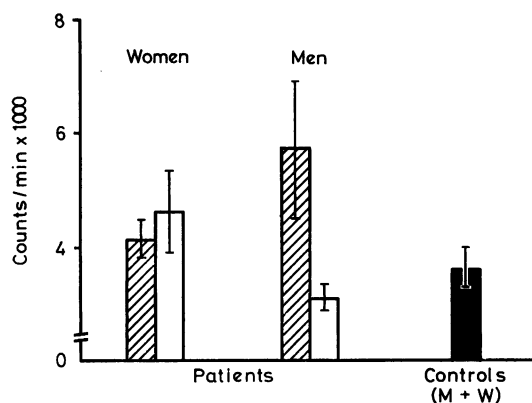


Figure Lymphocyte reactivity to T_2 in cell cultures from 21 female and 21 male patients with (hatched) and without (white) the presence of complement-fixing antibodies, and in cell cultures from blood donors (black). The bars represent the mean counts/min \pm SE in patients and controls.

Table 3 Induction of DNA synthesis by T_2 in lymphocytes from male and female patients with gonorrhoea and from blood donor controls

Male patients				Female patients			
Diagnosis	No. of patients	10 log counts/min \pm SE (antilogs within parentheses)	t values	Diagnosis	No. of patients	10 log counts/min \pm SE (antilogs within parentheses)	t values
Single infection	6	3.628 \pm 0.126 (4246)	0.04	Single infection	11	3.931 \pm 0.075 (8531)	2.38*
Multiple infections	8	3.553 \pm 0.206 (3572)	0.37				
Prostatitis	7	3.630 \pm 0.178 (4265)	0.05	Salpingitis	8	3.949 \pm 0.194 (8892)	1.76
Total patients	21	3.595 \pm 0.113 (3935)	0.16	Total patients	21	3.914 \pm 0.082 (8203)	2.40*
Controls (male and female)	18	3.622 \pm 0.090 (4187)		Controls (male and female)	18	3.622 \pm 0.090 (4187)	

t = statistical significance between the respective patient group and the control group

* = $P < 0.05$

was higher in cell cultures from seronegative women than from seronegative men (t value = 3.10 $P < 0.01$). A significant difference (t value = 2.76 $P < 0.01$) was also noted in the lymphocyte reactivity to gonococcal-antigen between the controls and all the patients except those men who gave negative results in serological tests. There was no difference between these two groups with respect to the thymidine uptake in NPN-stimulated cell cultures (t value = 0.78). The lymphocyte response to PHA and PPD was not significantly different in any of the above-mentioned groups and subgroups. There was no difference in DNA synthesis between the T_2 stimulated cell cultures from male and female controls.

Discussion

In the present study there were no demonstrable differences in the lymphocyte response to gonococcal antigen between the 42 patients with gonococcal urethritis and the 18 blood donor controls (Table 1). A comparison of this lymphocyte reactivity between the controls and each of the subdivided groups showed the same results. These findings confirm previous observations by Rosenthal (1972, 1975). One explanation of these results could be the presence of asymptomatic male and female carriers of gonococcal infection in the group of controls (Carpenter and Westphal, 1940; Pariser, 1972; Handsfield *et al.*, 1974). In previous studies on the lymphocyte response to gonococcal antigen in patients with chronic Reiter's disease, Rosenthal (1972, 1973, 1976) observed that 41% of the lymphocytes from controls gave an intensified cellular reaction in response to gonococcal antigen. Gonococci have many antigens in common with meningococci. Rosenthal and Danielsson (1978) found a close antigenic relationship between these microorganisms. Therefore the possibility of an immune response developing as a result of meningococcal infection cannot be excluded.

A significant difference (t values = 2.40 $P < 0.05$) in lymphocyte reactivity was noted only between female patients and controls (Table 3). It has been postulated that a persistent exposure to the gonococci results in a more intense antigenic stimulation (Hess *et al.*, 1965; Kraus *et al.*, 1970, 1975) which could be one of the reasons for a humoral and cellular immune response. With the lymphocyte-stimulation test, as with the serological test (Ratnatunga, 1971), a stronger response to gonococcal antigens is observed in women than in men. This could be because a larger area of mucous membrane is exposed to the infec-

tion. The duration and dose of infection may be greater in women because of the delay in diagnosis owing to lack of symptoms or to the presence of latent complications, for instance salpingitis. The duration of symptoms before diagnosis and sampling was short in all the six male patients with single gonococcal urethritis but only in two of 13 females with uncomplicated urethritis. In the male patients with repeated gonococcal urethritis the history of the present infection was also short (between one day and three weeks). It should be pointed out that except for two patients the others had had only one previous infection.

Surprisingly few sera from the group of patients with urogenital complications and from those with multiple infections gave positive results in the GCFT in our study, which contrasts with previous experience (Danielsson *et al.*, 1972). The sera from patients with prostatitis and salpingitis were obtained two years after the collection of the strains for antigen production, while the sera from patients with uncomplicated gonococcal urethritis were collected at the time of antigen production. The results of the serological analyses were obtained with the same antigen preparation comprising five strains. The lack of reactivity could be due to a change in the population of strains that cause infection or to the absence of antigenic determinants in the strains chosen for antigen production. Unfortunately, the gonococcal strain used to produce antigen for the lymphocyte-stimulation test was not available for the GCFT. In the absence of methods to select strains for antigen production, one has to rely either on single strains that have been proved to possess a broad antigenic spectrum (Torrey, 1940; Welch and O'Reilly, 1973) or on a collection of strains thought to be typical of the spectrum of strains present in the population under study. The importance of careful selection of strains is stressed by the finding that even in neighbouring areas there exist significant differences in the antigens to which the population is exposed (Sandström and Danielsson, 1977). In the present study we were able to demonstrate the presence of a lymphocyte response to gonococcal antigen in some but not all of the patients with gonococcal infection. Gonococcal antigen can be present in prostatic secretion three weeks after infection in 20–40% of patients with clinically healed gonococcal urethritis (Danielsson and Molin, 1971).

The lymphocyte proliferation test might be a new diagnostic parameter in patients with chronic and latent gonococcal infection, as humoral antibodies cannot always be demonstrated in these patients.

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References

- Bøyum, A. (1968). Separation of leukocytes from blood and bone marrow. *Scandinavian Journal of Clinical and Laboratory Investigation*, **21**, 1–109.
- Carpenter, C. M., and Westphal, R. S. (1940). The problem of the gonococcus carrier. *American Journal of Public Health*, **30**, 537–541.
- Danielsson, D., and Molin, L. (1971). Demonstration of *Neisseria gonorrhoeae* in prostatic fluid after treatment of uncomplicated gonorrhoeal urethritis. *Acta dermato-venereologica*, **51**, 73–76.
- Danielsson, D., Thyresson, N., Falk, V., and Barr, J. (1972). Serologic investigation of the immune response in various types of gonococcal infection. *Acta dermato-venereologica*, **52**, 467–475.
- Esquenazi, V., and Streitfeld, M. M. (1973). Transformation of lymphocytes in gonorrhoea before and after therapy. *Infection and Immunity*, **8**, 503–509.
- Grimble, A. S., and McIlmurray, M. B. (1973). Cell-mediated immune response in gonorrhoea. *British Journal of Venereal Diseases*, **49**, 446–449.
- Handsfield, H. H., Lipman, T. O., Harnisch, J. P., Tronca, E., and Holmes, K. K. (1974). Asymptomatic gonorrhoea in men. *New England Journal of Medicine*, **3**, 117–123.
- Hess, H. E., Hunter, D. K., and Ziff, M. (1965). Gonococcal antibodies in acute arthritis. *Journal of the American Medical Association*, **191**, 531–534.
- Kearns, D. M., Seibert, G. B., O'Reilly, R., Lee, L., and Logan, L. (1973). Paradox of the immune response to uncomplicated gonococcal urethritis. *New England Journal of Medicine*, **289**, 1170–1174.
- Kellogg, D. S., Jr, Peacock, W. L., Jr, Deacon, W. E., Brown, L., and Pirkle, C. I. (1963). *Neisseria gonorrhoeae*. I. Virulence genetically linked to clonal variation. *Journal of Bacteriology*, **85**, 1274–1279.
- Kraus, S. J., Brown, W. J., and Arko, R. Y. (1975). Acquired and natural immunity to gonococcal infection in chimpanzees. *Journal of Clinical Investigation*, **55**, 1349–1356.
- Kraus, S. J., Perkins, G. H., and Geller, R. C. (1970). Lymphocyte transformation in repeated gonococcal urethritis. *Infection and Immunity*, **2**, 655–658.
- Magnusson, B., and Kjellander, J. (1965). Gonococcal complement-fixation test in complicated and uncomplicated gonorrhoea. *British Journal of Venereal Diseases*, **41**, 127–131.
- Mishell, R. I., and Dutton, R. W. (1967). Immunisation of dissociated spleen cell cultures from normal mice. *Journal of Experimental Medicine*, **126**, 423–442.
- Pariser, H. (1972). Asymptomatic gonorrhoea. *Medical Clinics of North America*, **56**, 1127–1132.
- Ratnatunga, C. S. (1971). Evaluation of the gonococcal complement-fixation test. *British Journal of Venereal Diseases*, **47**, 279–288.
- Rosenthal, L. (1972). Cell-mediated immune response in patients with postgonorrhoeal arthritis, Part I. Paper presented at Medicinsk Riksstämman, Stockholm.
- Rosenthal, L. (1973). Cell-mediated immune response in patients with postgonorrhoeal arthritis, Part II. Paper presented at the XIII International Congress of Rheumatology, Kyoto, Japan.
- Rosenthal, L. (1975). Cellular immunity in gonococcal infections. In *Genital Infections and Their Complications*, pp. 261–269. Almqvist and Wiksell: Uppsala.
- Rosenthal, L. (1976). Cell-mediated immune response in uroarthritis. (a) The inhibition of migration of sensitized human lymphocytes by gonococcal antigen. (b) Lymphocyte transformation *in vitro* measured by ¹⁴C-thymidine uptake. *Scandinavian Journal of Rheumatology*, **5**, 209–215.
- Rosenthal, L., and Danielsson, D. (1978). Induction of DNA synthesis in lymphocytes *in vitro* by various bacteria, with special reference to *Neisseria gonorrhoeae*, in patients with uroarthritis (Reiter's disease). *Scandinavian Journal of Rheumatology*, in press.
- Sandström, E. G., and Danielson, D. (1975). A survey of gonococcal serology. In *Genital Infections and their Complications*, pp. 253. Almqvist and Wiksell: Uppsala.
- Sandström, E. G., and Danielsson, D. (1978). Studies on the influence of antigens on the results with the gonococcal complement-fixation test in patients with uncomplicated and complicated gonorrhoea. *Acta dermato-venereologica*, **57**, 547–552.
- Torrey, J. C. (1940). A comparative study of antigens for the gonococcal complement fixation test. *Journal of Immunology*, **38**, 413–430.
- United States Public Health Service (1965). *Standardized Diagnostic Complement Fixation Method and Adaption to Micro Test*. Monograph No. 74, Publication 1228. Department of Health, Education, and Welfare: Atlanta.
- Welch, B. G., and O'Reilly, R. J. (1973). An indirect fluorescent antibody technique for study of uncomplicated gonorrhoea. I. Methodology. *Journal of Infectious Diseases*, **127**, 69–76.